Biophysics Group(Annual Report)

journal or publication title	The science reports of the Tohoku University. Ser. 8, Physics and astronomy
volume	3
number	2
page range	156-159
year	1982-08-25
URL	http://hdl.handle.net/10097/25501

Biophysics Group

Associate Profeessor	Fumio Tokunaga
Assistant	Tatsuo Iwasa, Mikio Kataoka (from October)
Technical Staff	Yumiko Ito
Graduate Student	Mitsuhiro Hirai (D2), Masae Sato and Kazuo Takeda (D1), Satoshi Koike (M2), Osamu Hisatomi, Kazuhiro Nakajima and Masashi Takao (M1)

Research Activities

(I) Visual pigments (F. Tokunaga)

(a) Primary process of vision: Especially the relation between receptor potentials of visual cells and photobleaching intermediates of visual pigments (1)

Vision is triggered by photo-absorption of visual pigments. The visual pigments bleach and receptor potentials are generated from visual cells. The relation between the photobleaching of the pigments and the receptor potentials have been studied.

It was suggested that the first photoproduct hypsorhodopsin has all-*trans*-retinal and its thermal reaction is very small change in the structure. The early receptor potential was not directly but indirectly related to the intermediate.

(II) Bacteriorhodopsin (F. Tokunaga and T. Iwasa)

(a) Flash-induced fast change in surface potential and fluidity of purple membrane studied by spin label method (2)

Flash-induced changes in surface potential and fluidity of purple membrane were studied by a spin label method. Changes in surface potential and fluidity were monitored by observing the distribution of charged and uncharged spin labels between the purple membrane and the aqueous phase.

On flash illumination, a transient hyperpolarization of the surface potential and a transient fluidization of the membrane hydrophobic region are induced. The former may probably reflect the proton movement near the purple membrane surface, while the latter may result from photo-induced conformational changes of bacteriorhodopsin.

The two events are different in time course. The relationships between the two events, and the formation and decay of the intermediates of bacteriorhodopsin in the photoreaction cycle were discussed.

(b) Purple membrane analogs synthesized from C_{17} aldehyde (3)

All-*trans*, 11-*cis* and 9-*cis* isomers of the C_{17} aldehyde analogs of retinal bound with purple membrane apoprotein, probably through a Schiff base linkage at the normal retinal binding site. The complex formed from C_{17} aldehyde and purple membrane apoprotein was slowly decomposed by 10 mM hydroxylamine. The

C_{17} aldehyde competitively inhibited the regeneration of purple membrane from all-*trans*-retinal and purple membrane apoprotein. The differential ability of the different isomers to inhibit the regeneration suggests that purple membrane has a binding site for the side chain of retinal in addition to the Schiff base binding site.

(c) Photochemical reaction of 13-*cis*-bacteriorhodopsin studied by low temperature spectrophotometry (4)

The photoreaction cycle of 13-*cis*-bacteriorhodopsin (13-*cis*-bR) was investigated by low temperature spectrophotometry using two differnt preparations; 13-*cis*-bR constituted from bacterioopsin and 13-*cis*-retinal, and dark-adapted bacteriorhodopsin (bR^D), which is an equi-molar mixture of 13-*cis*-bR and *trans*-bR.

By irradiation with 500 nm light at -190°C, 13-*cis*-bR was converted to its batho-product, batho-13-*cis*-bR (bahot-bR^{13}), which is different from batho-product from *trans*-bR, batho-bR^t. On warming batho-bR^{13} to -5°C in the dark, it completely changed to *trans*-bR. We estimated the composition of 13-*cis*-bR and *trans*-bR in the warmed sample spectrophotometrically and then the absorption spectrum of batho-bR^{13} was calculated. The absorption maximum lies at 608 nm, 1250 cm^{-1} longer than that of 13-*cis*-bR; the molar extinction coefficient (ε) is about 74 000 M^{-1} cm^{-1}, larger than that of 13-*cis*-bR (52 000 M^{-1} cm^{-1}).

On the warming the sample containing batho-bR^{13} formed by irradiating 13-*cis*-bR or bR^D at -190°C, we could not detect other intermediates such as the lumi- or meta-intermediates seen in *trans*-bR system.

(d) The photoreactions and photosensitivity of 3,4-dehydro-bacteriorhodopsin at low temperatures (5)

The photoreaction of the artificial pigment synthesized from bacterioopsin and *trans*-3,4-dehydro-retinal, [3,4-dehydro]bacteriorhodopsin ([3,4-deH]bR^t) was investigated with low temperature spectrophotometry.

The amount of batho-product formed from the light-adapted pigment of [3,4-deH]bR (designated as batho-[3,4-deH]bR^t) by irradiation at 77 K was much less than that from *trans*-bacteriorhodopsin (bR^t) and depends on temperature at irradiation of the sample. The kinetics of photoconversion of [3,4-deH]bR^t to batho-[3,4-deH]bR^t and that of its reversion were measured at several temperatures with a so-called "double Dewar system". The results showed that the photosensitivity of [3,4-deH]bR^t was temperature dependent. When batho-[3,4-deH]bR^t was warmed above 143 K, it was converted to lumi-[3,4-deH]bR^t. Lumi-[3,4-deH]bR^t was also produced by irradiation of [3,4-deH]bR^t at 143 or 163 K. The maximum in the difference spectrum between lumi-[3,4-deH]bR^t and [3,4-deH]bR^t was located at about 540 nm. The irradiation of [3,4-deH]bR^t at 183 K produced an intermediate analogous to meta-bR^t, but under similar conditions bR^t does not produce meta-bR^t. These results indicate that intermediates of [3,4-deH]bR^t are less stable than those of bR^t.

The differences between bacteriorhodopsin and [3,4-dehydro]bacteriorhodopsin were discussed and compared with the differences between rhodopsin and [3,4-dehydro]rhodopsin.

(e) Photoreaction of the acidified form of bacteriorhodopsin and its 9-*cis* derivative in purple membrane at low temperatures (6)

The photoreaction of the acidified form of bacteriorhodopsin and its 9-*cis* derivative was studied by low temperature spectroscopy.

A short exposure of the acidified form of bacteriorhodopsin, which was prepared by adding 2 mM HCl to purple membrane suspension in 67 % glycerol at 0°C, to red light at -72°C resulted in the blue-shift of the spectrum. The feature of the shift was very similar to that accompanied by the formation of stable 9-*cis* acidified form of bacteriorhodopsin at 0°C, but only 13-*cis*- and all-*trans*-retinals were found in the extract from this product. No blue-shifted product was found on irradiation at -190°C.

Irradiation of the 9-*cis* form of acidified bacteriorhodopsin at -72°C with blue light caused the isomerization of its 9-*cis*-retinylidene chromophore to 13-*cis* and all-*trans* forms without a significant spectral change. It became greater only after the sample was warmed above -24°C. These results indicate the presence of the light-induced product which has *trans* configuration on the 9-10 double bond and exhibits the 9-*cis* type spectrum.

(III) Retinochrome (F. Tokunaga)

(a) Photochemistry of retinochrome (7)

Retinochrome is a photopigment found in the visual cells of cephalopods. It has been considered to act as a supplier of the 11-*cis*-retinal required for synthesis of rhodopsin, because its all-*trans* chromophore is isomerized to 11-*cis* form in the light. Light and thermal reactions of squid retinochrome were investigated by low-temperature spectrophotometry.

On irradiation with green light at liquid-nitrogen temperature, retinochrome (λ_{max} 496 nm, -190°C) is converted mainly to an intermediate lumiretinochrome (λ_{max} 475 nm, -190°C), its chromophore being changed to 11-*cis*-retinal. On irradiation with blue light at -190°C, retinochrome is changed to a photosteady-state mixture (λ_{max} 487 nm, -190°C) composed mainly of retinochrome and lumiretinochrome, since lumiretinochrome is partially regenerated back to retinochrome. Similarly, irradiation of lumiretinochrome with blue light also results in the same photosteady-state mixture, which can be completely reverted to lumiretinochrome on re-irradiation with green light.

Lumiretinochrome is stable at a wide range of temperatures from -190°C to about -20°C. Above -20°C, it is further converted, thermally, into metaretinochrome (λ_{max} 470 nm), which is the same bleached product as has been observed on irradiation of retinochrome at room temperatures. Thus, the light-bleaching process of retinochrome is rather simple compared with that of rhodopsin.

Publications

(1) Primary process of vision: Especially the relation between receptor potentials of visual cells and photobleaching intermediates of visual pigments, F. Tokunaga,

The Naito Foundation Annual Report, (1981), 63-65

(2) Flash-induced fast change in surface potential and fluidity of purple membrane studied by spin label method, S. Tokutomi, T. Iwasa, T. Yoshizawa and S. Ohnishi, Photochem. and Photobiol., 33, (1981), 467-474

(3) Purple membrane analogs synthesized from C_{17} aldehyde, F. Tokunaga, T.G. Ebrey and R. Crouch, Photochem. and Photobiol., 33, (1981), 495-499

(4) Photochemical reaction of 13-*cis*-bacteriorhodopsin studied by low temperature spectrophotometry, T. Iwasa, F. Tokunaga and T. Yoshizawa, Photochem. and Photobiol., 33, (1981), 539-545

(5) The photoreactions and photosensitivity of 3,4-dehydro-bacteriorhodopsin at low temperatures, T. Iwasa, F. Tokunaga, T.G. Ebrey and T. Yoshizawa, Photochem. and Photobiol., 33, (1981), 547-557

(6) Photoreaction of the acidified form of bacteriorhodopsin and its 9-*cis* derivative in purple membrane at low temperatures, A. Maeda, T. Iwasa and T. Yoshizawa, Photochem. and Photobiol., 33, (1981), 559-565

(7) Photochemistry of retinochrome, R. Hara, T. Hara, F. Tokunaga and T. Yoshizawa, Photochem. and Photobiol., 33, (1981), 883-891

Master Thesis (March 1982)

(1) Study on light energy bioconverter, bacteriorhodopsin — Importance of tyrosine residues in the conversion mechanism —, Kazuo Takeda.